Biotechnology I

Lecture by:
dr. F. Steinhäusler

Paris Lodron University of Salzburg
Austria

Table of Contents

Biotechnology lab - what goes on in industry and research.

Recommended Literature:

Journals: Key issues: links between labs and industry
- Biotech Forum (EU)
- Biotechnology Letters (UK)
- Brauwelt (GER)
- Biotechnology and Bioengineering (USA)

Books:
- Brock T.D. Biology of Microorganisms (USA)
- Griffiths A.J.F. et al., Genetic Analysis (USA)

HTML- addresses:
- http://washingtonpost.com/wp-dyn/health/specials/genetherapy/
- http://washingtonpost.com/wp-dyn/nation/specials/science/cloning/index.html

Part I - Definitions

Biotechnology is very interdisciplinary; engineering is incorporated with technology; it uses living organisms to carry out defined biochemical processes for industrial applications.

1. Stack (waste) effluent and gas concentration — [Vol %] e.g. CO_2
   stock concentration
2. Decay constant — Kd [s^{-1}]
3. Respiration coefficient — RQ [mol CO_2/mol O_2]
4. Efficiency (η): Background energy
   - Reference to substrate — $Y_{x/s}$ in [g dried biomas / g turn around material]
   - Reference to ATP — Y_{ATP} in g [dried biomas / mol turn around ATP]

   h refers to substrate or energy in form of ATP; another one would be oxygen; i.e. how much O_2 is required to produce one gram biomass — [g dried biomass / mol O_2].
   η can be also expressed with reference to the substrate electrons — [g dried biomass / (# reactive substrate e^-].
5. Biological O_2 requirement (**BSB_5**) — [mg O_2 / L·5d]
6. Chemical O_2 demand (**CSB**) — [mgO_2 / L]
7. Dextrose equivalent (**DE**) — [%]
   using glucose as a reference substance; mol reduction equivalent in substrate
   (ref. glucose = 100g / g dried biomass and hour)
8. Inoculation amount IQ — [%] or [L]
9. Michaelis Menten constant K_M — [mg/L]
10. Monod Constant K_MK_S — [mg/L]
11. Phosphate/Oxygen ratio P/O — [mol P / mol O]
    quantification of used mole of phosphate versus oxygen

Common denominator: Fermentation and Fermentation technology. How much biomass is produced for every mol O_2 used up in the process.

A. Scope of Biotechnology:

The lecture will cover the following topics:

a) products and services to the community.
b) not all biotech is new (fear of new technology).
c) advantage and potential disadvantages (based on today's perspective - theoretical risks).
d) future trends in biotechnology.
e) ethical, social, and economical implications by applying biotechnology in society.
f) new production methods in genomic science and its uses.

What is biotechnology: it uses living organisms to carry out defined biochemical processes for industrial applications; i.e. organisms, in the manufacturing or service industry, like genetic fingerprinting, gene therapy, etc.

Which branches are involved in biotechnology: Microbiology, Genetics, Biochemistry, Biophysics, Molecular Biology, Bioengineering, etc.

- **Biotechnology is not new,** it is based on ancient technology; for example, beer brewing was already known to the Babylonian's (6000 BC); even in mediaeval times, alcohol fermentation was practiced by the catholic monks, or in district distillations of ancient China;
- **Breadmaking skills**: the inoculation of fresh dough with one of the previous day was known to the ancient Europe, and even to Egyptians, some 4000 years ago (germination of barley);
- **Diary products**: the production of yogurt, cheese, kefir, and even vinegar was known for centuries; the monks were even familiar with the beneficial effects of these milk products;

Milestones in Biotechnology:

1897: Buchner Brothers - laid the foundation for enzyme technology. They isolated enzymes from yeast in a clear and scientific manner;

1929: Discovery of Antibiotics - many lives were saved during WWII because antibiotics were produced on a large scale.

1950 and onwards - technical advances like the discovery and use of the SEM and the TEM; biological discoveries and DNA research; in 20 years, around 20 Nobel prices were related to that scientific branch;

1970's -1980's - industrial applications were launched as the industry recognized the potentials and economical benefits and profits of biotechnology;

Fields of interest:

Even though many mistakes were made in the sectors of agriculture, medicine, and mining industry; the commercial logic behind it, is that it aims at a **cleaner** way to produce food, generate **medicines** that can treat new and old diseases; secondary environmental effects (as observed with oil spills) in which microbes are used in the cleanup process; all these beneficial effects seem to ignore the reverse side effects.

Skeptics:

Agriculture: Development of GM-foods and other alternative products raised concerns among the general public; secondary environmental effects due to the introduction of raw GM-products into the wild are poorly understood;

Industry in general: new, highly skilled jobs will be created, cheaper ways in manufacturing certain goods, current concerns about the clean-up of wastes could be talked with biotechnology.

General public: New products are viewed at with suspicion, new GM-foods are not always properly labeled; the public often doesn't even know what are the origins of a particular product; upon introduction into the market, certain aspects of a product are often not forwarded to the public;

Legislative body: laws regarding GM-products, how to regulate and control the new products are not existing, giving industry almost an open door to operate.

Why biotechnology is very attractive to industry:

1. It can utilize *waste products* as a raw materials to create new products; these raw substances are cheaper to produce than most traditional industrial products.
2. It requires very low energy requirements - most industrial applications operate at low temperatures; it does not need large power plants which makes it applicable for less developed countries; unfortunately, it can be used not only for the benefit of mankind but also as a tool in the "bio-warfare programs", for example.

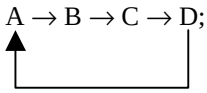
I.B. Basic principles of biotechnology - Genetic engineering:

Biotechnology modifies the cell's metabolism with the purpose of maximizing the production of desired metabolic materials.

- Scientists and bio-engineers *explore the biochemical steps of cells* in order to make particular products (by carefully selecting distinct cell-own enzymes);
  the cells metabolic machinery generates:
  i) energy,
  i) biomass, and
  i) degrades a particular substrate that it feeds on;
- At specific stages of the cell's lifecycle, when abiotic parameters change drastically, some protective changes in the microorganism (e.g. bacteria) modifies its life cycle, launching for example spore formation in bacteria, accelerated aging, build-up of antibiotics, etc.

To obtain a distinct product that is not generated by a particular organism require some changes and modification of the cell's DNA, that are followed by screening methods in order to select the one with the best qualities; a distinct gene-sequence is introduced into a third cell (represents a forcefully induced mutation) to trigger changes until the desired cell type with the looked for metabolic products is obtained. Genetic engineering is far more reliable than exposing the microorganisms to energy-rich electromagnetic radiation (X- or γ-rays); i.e. no longer mutations are brought about by random impacts, but rather directed by introducing a selected gene-sequence into the cells genome.

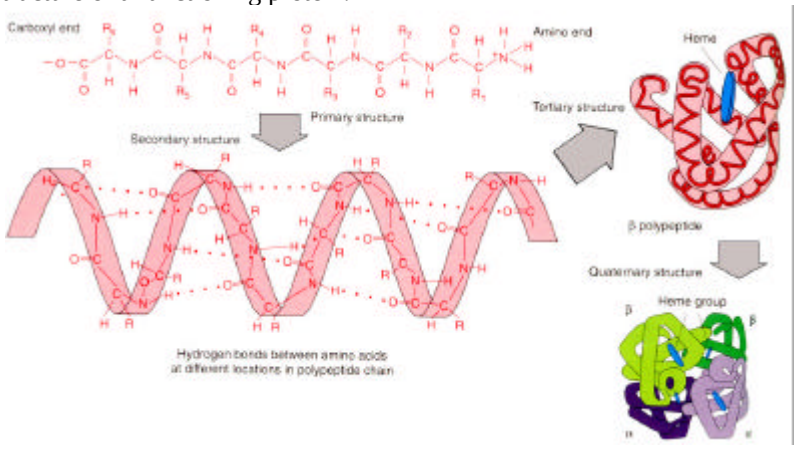
Basically, the **biochemistry of a cell** follows a metabolic pathway through a series of *intermediates* or in gaps which they are often used for the advantage of biotechnology.



Each intermediate product is a clear indicator to the biochemist in which a stage of a particular pathway the cell is working on. Biochemistry uses these intermediates to influence the production line by regulating and controlling the enzymes, i.e. the cell metabolisms by the way of the ***negative feedback loop***:
The output "D" is used to control the amount of e.g. intermediate "B"; the cells are modified in a way to lower the losses of intermediate reactions in order to maximize the desired end product "D".
Besides being self-regulated, the negative feedback loops have the advantage of avoiding waste of energy and the sudden stop of production. A single gene controls a particular characteristic; therefore, genes responsible for a previous intermediate influence of subsequent steps that depend on its products.

Flashback: During WW II, it was essential for Germany to import vegetable oils to obtain glycerol for the production of explosives (nitroglycerine). Britain, the enemy of Germany simply stopped the orders that left from the UK; in order to keep up with the high requirement of glycerol, the Germans found out that the yeast of soy beans can be used instead: Exposing yeast cells to hydrogen sulfide (H_2S), the microorganism started to generate glycerol as a byproduct. On the other hand, Britain was strongly reliant upon acetone imports from Germany to produce ammunition. As a counter measure, Germany blocked the export of acetone. British scientist ultimately found an alternative by exploiting the fermenting capacities of *Clostridium* sp.; an undesired byproduct of this fermentation was obtained in the form of H-gas - far too often that gas was the cause for violent explosions that destroyed the entire production facilities.

Gene & Protein: The gene is also the basic unit of heredity. A gene is a sequences of DNA that codes for the sequence of the primary structure of a protein. Single gene sequences within the genome, ultimately work together to produce the quaternary structure of a functioning protein.
The tasks of bio-engineers are easily outlined - they localize the genes that code for new enzymes and introduce them into a suitable host; i.e. the introduction of the new genes interfere with the already existing pathway by forcing the microorganism to generate substitute products; by doing so they assign that organism a totally new set of characteristics, besides creating species with new properties.



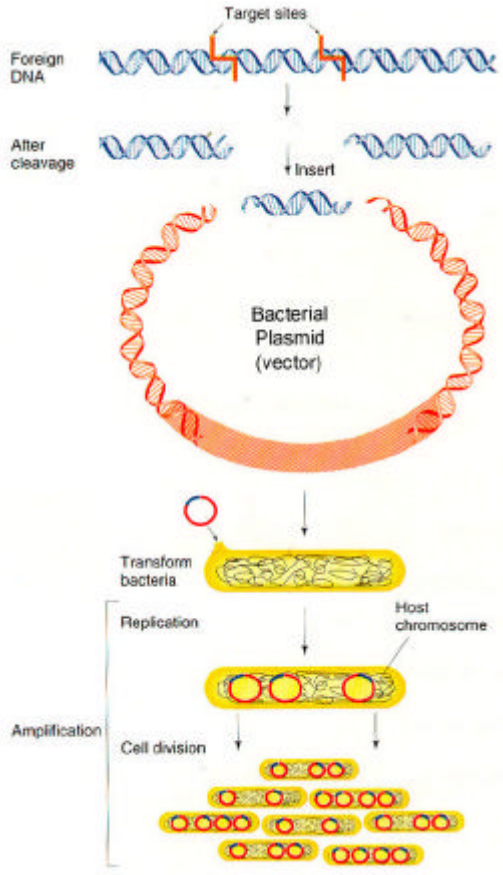
How its done? In the early 1970's ***restriction enzymes*** were discovered. These are naturally occurring enzymes that are part of the natural defense mechanisms that a bacteria use to defend themselves. When a bacteriophage (virus) infiltrates a bacterial cell, the bacteria release restrictions enzymes that cut the DNA/RNA of the invader in small fragments; consequently the viral DNA is not able to launch the reverse transcriptase in order to use the bacteria's own enzymatic machinery to produce more viruses. So far, many different restriction enzymes have been identified. Each restriction enzyme "cuts" at specific sites within the genome of an intruding DNA; Gene technology uses practical modification of these enzymes.
The basic tool to modify an organisms genome is achieved with recombinant DNA.

Recombinant DNA: Recombinant DNA is made by splicing a foreign DNA fragment into a small replicating molecule (such as a plasmid), which will then amplify the fragment along with itself resulting in a molecular "clones" of the inserted DNA fragment.

Making Recombinant DNA: The organism under study, which will be used to donate DNA for the analysis, is called the donor organism. The basic procedure is to extract and cut up DNA from a donor genome into fragments containing one to several genes and allow these fragments to insert themselves individually into opened-up small autonomously replicating DNA molecules such as bacterial plasmids. These small molecules act as carriers, or vectors, for the DNA fragments. The vector molecules with their inserts are called recombinant DNA because they represent novel combinations of DNA from the donor genome (which can be from any organism) with vector DNA from a completely different source (generally a bacterial plasmid or a virus). The recombinant DNA mixture is then used to transform bacterial cells, and it is common for single recombinant vector molecules to find their way into individual bacterial cells. Bacterial cells are plated and allowed to grow into colonies. An individual transformed cell with a single recombinant vector will divide into a colony with millions of cells, all carrying the same recombinant vector. Therefore an individual colony represents a very large population of identical DNA inserts, and this population is called a DNA clone.

"**Cloning** allows the amplification and recovery of a specific DNA segment from large, complex DNA sample such as a genome".

Isolating DNA: The first step of making recombinant DNA is to isolate a donor and a vector DNA. General protocols for DNA isolation were available many decades before the advent of recombinant DNA technology. Using such methods, the bulk of DNA extracted from the donor will be genomic DNA, and this generally is the type required for analysis. The procedure used for obtaining vector DNA depends on the nature of the vector.



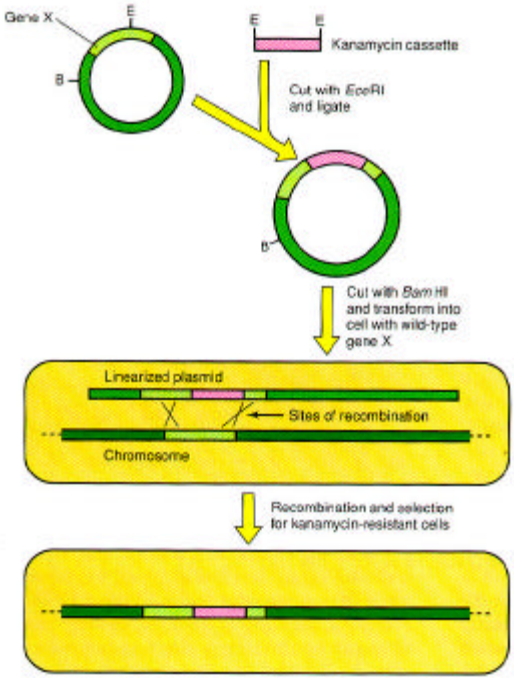
Bacterial plasmids are commonly used vectors, and these must be purified away from the bacterial genomic DNA.

Plasmids such as those carrying genes for resistance to the antibiotic can be separated from the bacterial chromosomal DNA. Because differential binding of ethidium bromide by the two DNA species makes the circular plasmid DNA denser than the chromosomal DNA, the plasmids form a distinct band on centrifugation in a cesium chloride gradient and can be separated easily. They can then be introduced into bacterial cells by transformation.

Restriction enzymes have two properties useful in recombinant DNA technology. First they cut DNA into fragments of a size suitable for cloning. Second, many restriction enzymes make **staggered cuts** generating single-stranded **sticky ends** conducive to the formation of recombinant DNA.

This type of segment is called a DNA palindrome, which means that both strands have the same nucleotide sequence but in antiparalled orientation. The enzyme cuts within this sequence, but in a pair of staggered cuts between the G and the A nucleotides, which means that fragments produced from a double strand DNA-helix have "**sticky ends**"; i.e. single stranded DNA with sticky ends have a sequence which enables self-recognition to pair with one another.

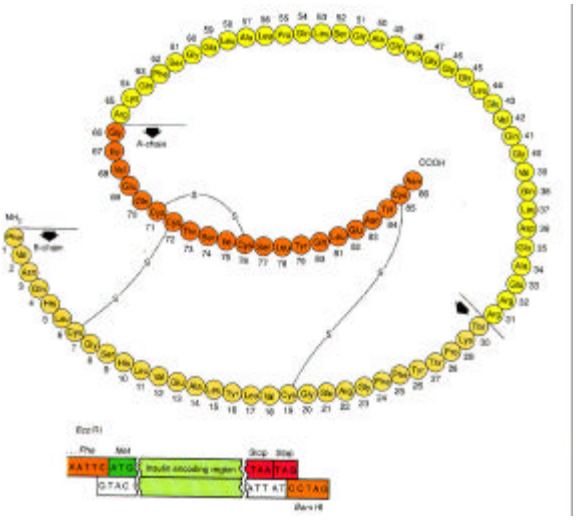
For example:
The restriction enzyme **EcoR1** recognizes the base-sequence G*AATTC*; it cuts the double stranded DNA into a AATT and TTAA
HindIII is an other restriction enzyme; it acts specifically on *AAGCTT* yielding single stranded fragments with AGCT and TCGA (of the mirror like strand) ends



Upon bringing these spliced strands together, they spontaneously join and form a double stranded DNA-segment.
DNA Ligase ("sealant") is important in the cutting of the same enzyme. Therefore, nature has been using this tools and uses them well all the time (in prokaryota, eukaryota, etc.); the methodology is not new only the endpoints.

Joining DNA: Donor DNA (sometimes called Foreign DNA) and vector DNA are digested with restriction enzymes and mixed in a test tube in order to allow the ends to join to each other and form recombinant DNA. There are several ways of joining the donor to the vector to create a recombinant DNA molecule.
Cleave DNA at a specific sequence and make single-stranded sticky tails. Such strands in the donor DNA then anneal to sticky ends in the vector, which has been cleaved by the same restriction enzyme.

Amplifying Recombinant DNA: Recombinant plasmid DNA is introduced into host cells by transformation. Once in the host cell, the vector will replicate in the normal way, but now that the donor DNA insert is part of its length, the donor DNA is automatically replicated along with the vector. Each recombinant plasmid that enters a cell will form multiple copies of itself in that cell. Subsequently many cycles of cell division will occur, and the recombinant vectors will undergo more rounds of replication. The resulting colony of bacterial will contain billions of copies of the single donor DNA insert. This set of amplified copies of the single donor DNA fragment is the **DNA clone**.
This **gene cloning** is a reproduction process (in DNA culture) that yields many copies of a single gene sequence. A **clone** is a group of cells, organisms, genes, etc. that are an exact copy of each other. Recombinant DNA is put together to generate many clones of a particular sequence.
The key element in gene cloning is the **plasmid**. It is a circular strand of DNA, that is separated from the bacterial chromosome – it is a natural vehicle that is often used to introduce a foreign gene into another cell - plasmids are a commonly used in transferring resistance genes against antibiotics from one cell to the other - this process is natural. As bacteria are R-strategists, they yield immediate results as they multiply quickly - the only task the researcher has to do is to find the cell with the wanted for properties.



How Biotech has transformed the industry: Advances in microorganisms are so efficient nowadays, that not only high yields are obtained, but also screening and selection of traits are limited to random checks.
Advantages: by adding faster synthesizing machines into a slow working host cell, the metabolic processes and the activation of key enzymes are boosted significantly. Once the proper cell density is reached, the production can get started.
Disadvantages: Microbial activity results in the production of substantial amounts of heat; it is necessary to provide cooling equipment in order to avoid overheating, which would ultimately terminate the bio-reaction. Cooling systems of fermentation tanks are very expensive. However, substituting genetically modified thermophobic microorganisms with thermophilic ones can help to reduce costs in cooling equipment.

I.C. Social implications - Who is afraid of biotech?

Legal situation, risks and release of patents: recently, the USA introduced a new maize strain (**BT**); its growth performance has been modified in a way that it produces a poison against all bugs that feed on it. Although this type of maize promised not to be dangerous to the environment, a recent field trial revealed that the death rate of the monarch butterfly, which feeds on it has well jumped to 44% upon the introduction of the new strain (article published in *Nature*).
As a consequence, the EU banned the release of any genetically modified food product.

A **biodiversity treaty** was drafted in Rio de Janeiro in late 1992; it outlines the principles of protection of flora and fauna; as a major goal it implies a stop to the ongoing trend of extinction.
So far the *US Natural Academy of Sciences* concluded that selective breeding did not harm diversity in crops, plants, grass, etc.; extrapolation of this conclusion to the GM sector is not possible since the term breeding is expanded to interspecies crossing!
e.g. selective breeding was successfully executed with prairie grass; a wild strain that was resistant to 40-45°C was crossed with a tropical grass that has higher yields; nowadays with biotechnology, the genes of heat resistance are extracted from that species and inserted into a non-related the target organisms.

Adhoc Team: in 1998 a team of scientists of about 100 different organizations (EPA, WWF, etc) met to discuss GM-products. Within a 6-month period, they were asked on behalf of the UN to come up with a conclusion regarding the potential impacts of GM-products (organisms and food products alike) upon biodiversity; they stated that ratification of the treaty is possible, but it cant be excluded that biotech will not have a detrimental effect upon natural plants and animals (potential effects of GM-products are not known - today the word "*potential*" is changed for "*can*" hurt biodiversity).

Laws and patents - "Can we pay for life"?
Advances in biotech are always ahead of the legal and conceptual system – this includes also public perception; therefore, the legal and the public do not have the answers.

A **patent** represents the exclusive right to manufacture or use a product. When scientists invent a new process or product, neither the scientists nor the company has the exclusive rights; unless, they apply for and be granted a patent. Such a product must meet certain qualifications, in particular it must be sufficiently different to already existing ones (both in output and method of production); in the case of particular properties of microorganisms, it must be able to degrade matter that was previously not used up by the organisms metabolism.
The **Drawback** in filing a patent is evident, at the moment of applying for the patent, the scientist or the company must supply descriptive and detailed information of the product before legal protection is given; therefore, many companies refrain from applying for a patent and try to keep their product contents secret.
Legal situation: In 1987, the USA legally paved the way for biotech-companies to patent life forms other than microorganisms.
In 1988, the USA patented its 1st ***transgenic mouse*** - used for standardized cancer research; likewise, a recently patented GM-monkey named ***Andy*** will be used to deepen the knowledge about diseases like diabetics, alzheimer's and breast cancer.
In Germany, the transgenic bull ***Herman*** was born (Gene Farming Europe); it carries a modified version of the protein **lactoferrin**, a natural defense substance against infections of the mammalian gland; furthermore, *Herman* passes this gene to its female offspring, making them less susceptible for *mastitis* (inflammation caused by the milking machine) and as a positive side effect give higher yields of milk. Since the breeding was carried out in the NL, while the animal is kept on a German farm, national laws apply only with restrictions. So far the EU-Parlament does not know how to deal in this particular case; patenting laws in EU lack behind those of the US; therefore, *Herman* could not be patented in EU.
Problem: who gets the royalties for every calf that originates from *Herman* and what will happen when the bull (or at least his sperm) eventually goes abroad?

Ethics: Is it morally justified to modify an animal for the sole destiny to benefit humans? The transgenic mouse was created to suffer in order to obtain a standardize test animal for cancer research.
Is it also morally justified to change the genetic diversity of our ecosystem?
Should the consumer know what a GM-product is made of (labeling of all GM-products)?
Perceived and actual threat:
i) recombinant DNA is as ordinary as any other DNA; i.e. such organisms aren't more dangerous as ordinary, naturally occurring ones - even though they might upset existing equilibria;
i) public perception generally condemns GM as potentially dangerous – as with any new technology....

I.D. GM organisms released into the environment: GM-bacteria are widely used in the pharmaceutical industry – mostly in fermentation processes. But what about to the occupational exposure to the workers for the dangers of spills, leaks, exposure to the GM-organism and its metabolic products while handling them?

1. The **safety code** is similar to those used in the nuclear industry; the use of high tech is necessary to prevent accidents - even though there is a very low rate of risk.
2. **Killing GM** organism at the end of the production cycle: during the cold war, the USSR ran its secret chemical warfare program that lasted till 1993; disposing off the toxic material proofed to be very difficult; the soviets dumped the substances along with concentrated acids and other toxic chemicals into several drilling holes at an island in the Aral sea. In 1995, The Kazakhstan government asked the US department of Health to check those sites – to the surprise of everyone, some cultures survived the chemical attack and were still found to be functional (**Biohazards**, by author Alibek Ulak).
3. ***E.coli* and yeast** are the most commonly used microorganisms in biotechnology. Neither of these organisms are capable to survive in the GIT of humans, but there is concern of deliberately releasing GM microorganisms into the environment. For example:
   - *Frost protecting microorganisms* that were designed for the agricultural industry to protect crops from frost damages. These crops contain the ice-nucleation gene; this gene is potentially dangerous, as it initiates ice formation at temperatures around –1.5 to –5°C (membrane mediated proteins are responsible for this); once this modified bacteria is released into the environment, scientists do not know if wild strains of this microorganisms will acquire this gene via bacterially mediated plasmid transfer.
   - So far, the ski-resort industry utilizes *non-modified kryophilic bacteria* to generate artificial snow; what might happen if they switch to GM-strains?
4. **Transportation**: Many GM-bacteria are sent via mail across the globe. If these strains are involved in an accident, there will be an uncontrolled release into the environment; the microorganisms will proliferate everywhere and there is absolutely no way to control it.

Such potential risk motivated scientists to incorporate ***suicide genes*** into the bacteria in order to trim down survival of the strain outside the controlled containment.
In either way, rigorous research requires to be done because mutations, hybridization with wild forms, and exchange with other bacteria might alter otherwise harmless strains.
Potentially high risks are evident for bacterial sterilization strains, like those ones intended for the extermination program planned to eradicate the rabbit population in Australia, or the mutant strains of small pox that so far resist any immunization attempts.

Competition with natural species:

- Better fitted GM microorganisms will probably outcompete the wild strains - how can such an event be monitored? The aim of GM products are to be different, produce higher yields, larger product-biomass, etc.; at the same time these organisms are highly resistant to several ambient factors – a classical event (though non-biotechnically modified) is already evident in the seaweed *Calerpa taxifolia*; it has been accidentally spilled into the Mediterranean sea - so far no remedy has been found to eradicate this mutant. On the other hand, GM-mammals are a lot easier to handle, as their reproductive DNA is passed on only via a suitable fe/male individual.
- **Using cloning vectors**: disease transmittance may be facilitated in microorganisms; especially antibiotic resistance genes are readily passed on via plasmids (conjugation of bacteria); likewise, usually non-toxic strains may catch up the toxic properties by the same way.
- **GM herbizide** resistance genes are used in plants to increase their production yield and raise resistance to predators; bioaccumulation seem to be a serious problem; scientists suggest that initial trials to generate such plants should be halted, as acting metabolites accumulate along the food chain; their use is cut down, even though many farmers want to increase their market share.

New drugs: new vaccines and drugs will be developed in the years to come; probably, these remedies will be "*a lot safer"* than conventionally produced ones; e.g.

i) Creutzfeld-Jacob-Disease (CJD) is "*definitely"* transmitted by the similar looking prion of BSE (bovine spongiform encephalitis) that occurs in natural growth hormones – synthetically generated hormones would have made transmission impossible;

i) BST (Bovine Somato Trophin) is a hormone that causes cows to give 25% more milk and is widely used in US-agriculture; unfortunately, the use of it implies severe side effects (see script p14);

Gene therapy – in short: extract a tissue sample, treat it and place it back to the organism.

Somatic gene therapy uses ordinary body cells; viruses contained in body cells are used as vectors (mediators) to introduce genes (oncogenes) into somatic cells to obtain the desired mutation; potential applications of this technique involve cancer therapy; i.e. latent cells that might trigger cancer. Since somatic cells are not handed on to offspring, this therapeutical approach seem to be far less controversial than germline gene therapy.

- **Skin graft**: GM-modified human skin cells that are obtained from the owner and placed back after they have sufficiently multiplied. The advantages of these skin transplants are not only used in victims who suffered burns, but also by introducing protein-encoding genes into the skin transplant that generate a missing substance required in patients suffering from diabetes, etc. Once these transplanted cells die, the beneficial production of those cells ceases as well.

Germline gene therapy involves forcefully induced mutation into genes of hereditary chromosomes of the reproductive tract - i.e. of sperm and eggs; misplacing a gene under that approach includes extremely high risks of causing birth defects or even the death of the resulting embryo or fetus;

- **Eugenics** allows biotechnology to selectively breed humans with particular properties; even though this branch implies great beneficial potentials – like the eradication of hereditary diseases – misuse can easily trigger the reverse effect. This branch of gene technology is viewed with great interest by the insurance industry and the military.

Economics – who will benefit from biotechnology? Moneywise the industrialized countries like FRG, CH, UK USA, etc. will benefit from this technology; some examples shall make it more obvious:

- **Sugar**: biotechnology brought major changes to the sugar industry; sugar producing countries like the Philippines suffered tremendously;
  HFCS (High Fructose Corn Syrup) is obtained from corn starch; it is 150% sweeter than conventional sugar; the soft drink industry in the US widely uses this substitute. So far the EU did not approve its use, as this would upset the current agricultural concept of the union.
  Aspartame is a completely artificial substitute; besides being 200 % sweeter than normal sugar it does not have any calories. As a result, the USA stopped sugar imports altogether, causing havoc to the Philippine sugar industry.
- **Rice**: Rice production is improved by N-fixing *Cyanobacteria*; introducing modified *Cyanobacteria* further increased the fertilizing effect giving India, Indonesia, and other countries the possibility to stop rice importation all together; as a result Pakistan suffered enormously.
- **R & D Sector**: Research and development benefits greatly from biotechnology, amongst other effects it:
  i) creates new skills and new jobs;
  i) improved quality of life due to progresses in health care;
  i) increased yields in agriculture;
  i) missing raw materials in old world can be produced by implementing GM-microorganisms that facilitate cost-effective production of these materials; e.g. the Carlsberg brewery encourages its farmers to grow raw materials that can be used in the textile and paper industry.

Part II - Industrial production of GM-products

Certain aspects regarding large scale production have to be taken into consideration:

- How to maintain production
- Scale of production
- Setup and auxiliary (lab, etc.) facilities for industrial application
- Practical application – e.g. industrial production of antibiotics

Safety consideration in biotechnology: any biotechnological application on a large scale production requires profound knowledge of system processes in order to avoid financial losses; i.e. strict guidelines are implemented to make sure that these processes run according to the designed pathway. Some of these safety considerations are as follows:

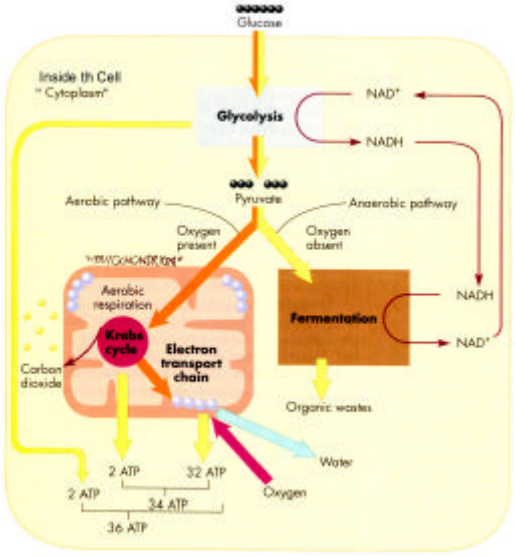
i) walls, floors, or any smooth surface must be kept **aseptic** at all times;

i) designated clean zones with full **protective clothing** are required to prevent any escape or contamination of cultured media and/or the system process itself, by or to the staff;

i) maintaining a **negative atmospheric pressure** gradient within the plant; laminar flow cabinets are used as well to prevent particles to vent out into the environment;

Fermentation: It is a chemical act or process, caused by a fermenting agent, of converting a carbohydrate into alcohol, acids, and other compounds, as yeast converts the sugar in grape juice into alcohol, producing wine. The ***fermentor*** is a tightly sealed reaction chamber in which a controlled reaction can take place. It keeps any contaminants (biotic and abiotic alike) from disturbing or even spoiling the enclosed reaction mechanism.

A strict definition of fermentation states that it is an anaerobic catabolism in which an organic compound serves as both an electron donor and an electron acceptor and in which ATP is produced by substrate-level phosphorylation (e.g. Emden-Meyerhof pathway of glycolysis).



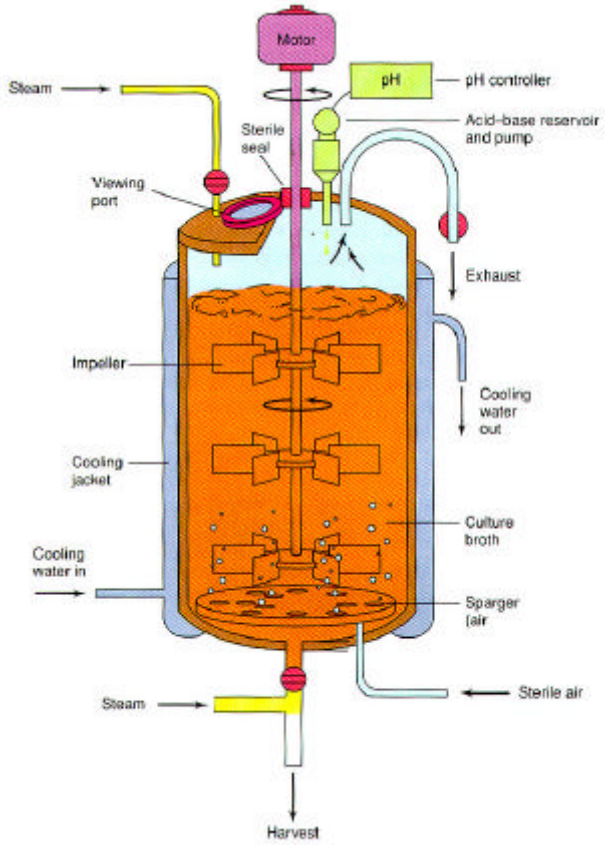
Design features and key aspects of a fermentation process: Keeping out unwanted microorganisms that might interfere with the production – sterility is the key word in biotechnology.

- Double mechanical seals
- Filters on exhaust pipes
- Sampling outlets; safety precautions at safety outlets (outlets are considered the weakest point in the system - spills are very common);
- Aerosol prevention; aerosols are perfect carriers of contaminants from/to the environment – especially if aerobic microorganisms are involved in the production process (O_2 encourages growth); aerosols can transport contaminants over large distances; off site contamination of people so far has not yet occurred;
- Effluents: maintaining aseptic conditions at all stages (continuous sterilization) requires a multiple cleaning process; this is partly achieved with on site installed killer tanks that contain toxic chemicals.
- Heat exchangers; the fermentation of microbial matter generates a substantial amount of heat; maintaining optimal temperature conditions is necessary to keep the system process going; if the cooling system is not properly set up, an excessive down-cooling mechanisms may cause the fermentor to burst (freezing).
- Centrifuges are notorious for leakage and aerosol generation; therefore, air-tight seals must be used.
- Vents require microbiological filters in order to avoid any escape of GM-organisms into the environment.
- Pipes and tubing - connective equipment must be leak proof;
- Integrated sterilization system for the entire factory – usually a super heated steam system is used to flush and heat-sterilize the pipes, tubes, and the fermentor; insertion of *in-situ* hot steam as some pockets are hard to reach. This also requires an aerodynamic design and polished surfaces of the tubes (in the experimental lab-phase, such expensive means are not required).

II.A. Running such a facility - Aseptic Operations:

Successful fermentation means zero contamination; for every item that is used, it must be sterile before inoculation. Practically this implies that:

- Fermentor, auxiliary equipment plus medium must be sterile;
- Sterilization of air-supply (HEPA filters); bacteriophags cannot pass such narrow filters (except nano-bacteria); a common practice is to heat and subsequently cool down the air before it passes the filter; - nothing must come in or leave the plant via the venting system - gases must be monitored carefully. Another aspects that has to be taken in consideration – the plant should not be located at a site where: i) persistent winds redirect exhaust gases towards densely populated areas (smells cause problems). i) farming communities should not be located nearby as contaminated aerosols my disrupt the production facilities (i.e. *E.coli* grows and reproduces perfectly in soils).
- Regular checks for mechanical micro-fractures;
- **Medium culture sterilization**; large scale production goes beyond the petri-dish or laboratory Erlenmeyer flask; it often reaches volumes of 1000's L; sterilization of the medium is done by passing the medium through coils that are exposed to super-heated steam; exposure time is crucial to avoid decomposition of the involved medium; such complex preparation often requires an extension of the production plant which only takes care about the sterilization tasks (doubling of costs).
- Once these crucial points have been checked, inoculation can take place;
- Foam formation: everything that agitates creates bubbles; therefore, antifoaming additives are necessary to avoid excessive foam formation; these additives have to be sterile as well**!**



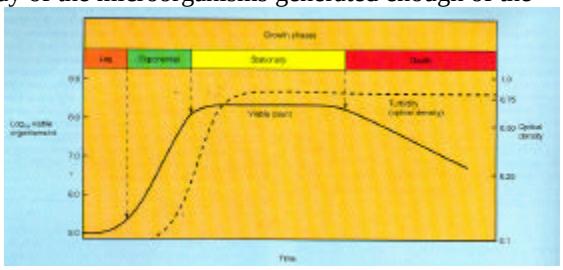
Picture of a fermentor:

The weakest point of a fermentor is the rotating interface of the motor and the rotor block that reaches into the fermentation chamber; specially designed bearings and lubricants are necessary to maintain sterility of the reaction chamber.

Fermentation can occur in various methods:

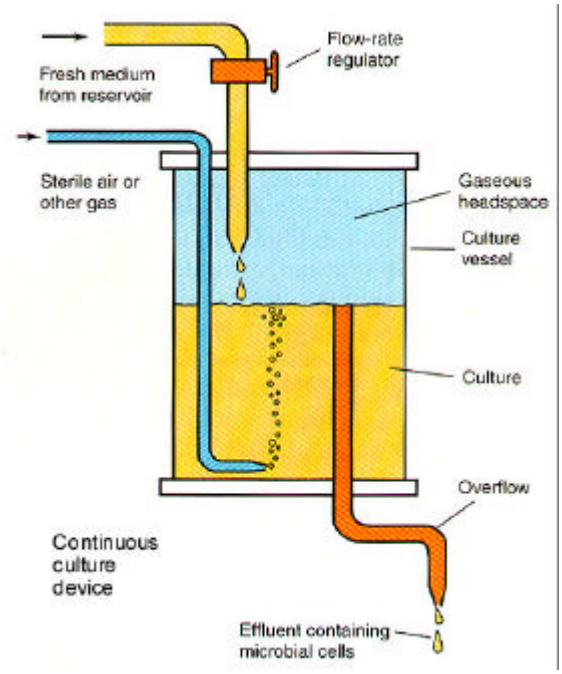
1. **Solid substrate**: since ages, the use of a solid substrate is one of the most common; i.e. mushrooms grown on a solid substrate, bread and cheese making is done with a solid substrate; microbial-mediated methane production from landfills, etc.
2. **Aqueous substrate**: fermentation that involves a liquid solution (mostly water):

- **Batch procedure**: Nothing is added or removed in this process, except venting of reaction gases. This is the simplest form of aqueous fermentation in that both the nutritive medium and the inoculated culture are left under preset conditions until the metabolic pathway of the microorganisms generated enough of the desired end product; at this stage the product is separated from the rest and processed further, while the microbial sludge is disposed off properly; i.e. incinerated.
  Diagram: all nutrients are used up during the fermentation. Internal environmental factors change gradually, nutrient supply, reaction temperature, metabolic waste concentration, etc.



Fed Batch: a slightly modified procedure; here nutrients are added at defined timing intervals; this guarantees a continuos production until the fermentor's holding capacity is reached (a fine tuned system).

- **Continuous procedure**: The addition of nutrients at a constant rate requires the extraction of product substance at the same rate. Continuos fermentation can only work if the reaction temperature, pH, O_2-concentration, and several other parameters are carefully monitored;



Advantages of this fermentation is the continuous production of end-material.

Disadvantages: It is a complex systems; if one parameter breaks down, the whole production collapses, generating considerable amount of waste; furthermore, cells tends to clump and clog in inlet/outlet pipes and foaming is another complication.

Setting up an industrial process: implementing a new production line requires three elementary steps;

1. **Basic steps**: At this stage the biotechnologist works with lab-flasks, petri dishes, screening techniques, determination of optimal growth parameters, harvest, etc. handling is generally very easy; the average volumetric sample circles around 200 cm^3 while the energetic demands fluctuate around ≈ 1 kW.
2. **Pilot plant**: this stage involves the construction of small scale fermentors (50 - 300 L); at this stage the engineers try to find out how upscaling affects reaction conditions compared to lab conditions.
3. **Large scale production facility**: A large plant is far more complex than a pilot plant; volumes of up to ≈ 10^3 L have to be handled properly, energetic demands, an appropriate cooling system, aseptic conditions for the entire factory, a constant flow of large volumes, etc. must be handled precisely. Upon termination of a particular fermentation process, harvesting means filtering, drying, distilling, and extracting, besides wasting 100 kg's in byproducts. A single error can result in the total loss of both culture and medium which will force the plant out of operation for days, weeks or even months.

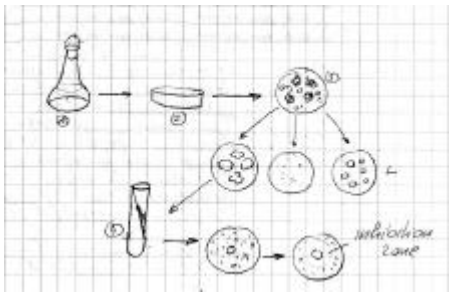
Practical example: When Flemming discovered antibiotics in 1929, he was basically working with penicillin of a *Potatum* contaminant in petri dishes. It was a very inefficient process; all instruments were unsuitable for large scale production, but in those 70 years biotechnology has made great advances; although, large scale industrial antibiotic production has not really taken off before WWII.

Even though antibiotics are entirely natural products, scientists have to admit that they are not quite sure why microorganisms produce them;

- a good explanation seem to be interspecific competition – but this is only part of the entire story;
- antibiotics are secondary metabolites; once the microorganism runs out of nutrients, it is capable of breaking them down again (via an enzymatic cascade) to overcome a nutritional short supply;
- production of an antibiotic is a metabolic waste product, besides being self-immune it keeps away competing organisms;

Processing an antibiotic: biotechnologists induce mutations by inserting extra-species gene sequences while; screening procedures enable the scientist to isolate the correct mutant;

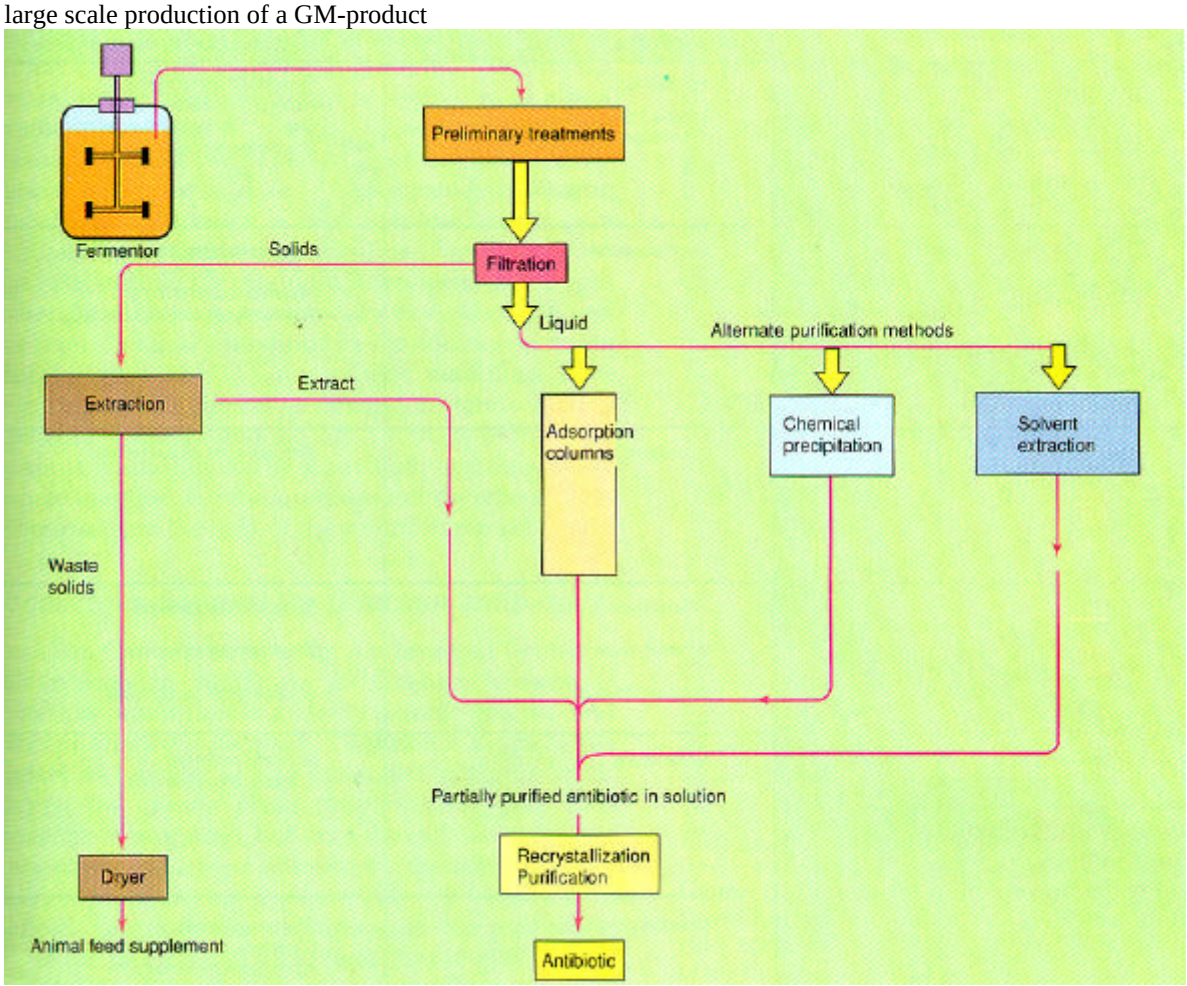
How to make antibiotics:



1. sample with microorganisms are kept in Erlenmeyer flask;
2. aseptic transfer of roughly 1 cm^3 onto a petri dish;
3. drying and incubating for about 48 hours is followed by selecting an individual colony and transferring it to several sterile petri dishes; further incubation is required;
4. a single colony of the unknown organism is transferred to an agar slope and incubated still further ....
5. screening by extracting a sample with a selected contaminant that is sensitive to a particular antibiotic - results in a zone of inhibition once it is exposed to it ....

Test for optimal growth, pH, temp, nutrient, # of products produced (chromatographic methods) new variations from old ones.
Every year the biotech industry discovers many new antibiotics, but only a few of them are further analyzed; as it goes to the market; a strict criteria must be met – if there are already an a-biotics that work efficiently, the industry doesn't feel the urge to process further alternatives;
In biotech industry continuos fermentation procedure is not often applied, as it requires a more complex peripheral machinery; because of the huge dimensions of the fermentors (holding capacity of 100,000 L or more) the most common method of production is done according to batch procedure with the usual control parameters (pH, temperature, nutrient levels, etc.) with aerobic fermentation.
Once the desired product concentration is reached, the product is extracted, the microbial sludge is filtered sterilized and further used as a supplement food in the agricultural industry.
Biotechnologically produced antibiotics are chemically modified strains of penicillin – often referred to as F/GIH.

large scale production of a GM-product



Part III - Biotechnology and Food production

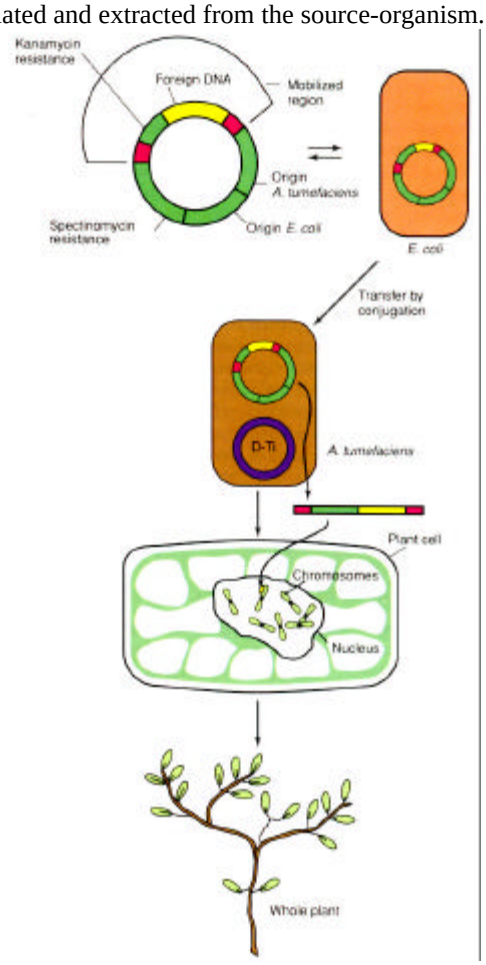
Topics: i) how Biotech is used in agriculture
i) pest-resistance in crops
i) BST and the turbo-cow
i) new foods obtain via microorganisms
i) byproducts of biotechnology as new food sources
i) biotechnology and fruit-juices

Biotech and **Agriculture**: Since its introduction, the biotech industry promised improvements of crops yields, resistance against diseases, enhancement of N-fixation, and improvement in shelf live of processed products.

In the **Animal sector**, the biotech industry tried to enhance productivity via the use of modified hormones.

Selective cross breeding was common before the advent of biotechnology – it was only possible to cross for example one weed with another – according to the recessives of the genes, the result of the hybrid was by then unpredictable.

- ***Treating weeds*** with x-rays or aggressive chemicals generally induced uncontrollable mutations, which resulted in a wide range of diversification in their offspring; such treatment is a rather wasteful process that takes a long time and is quite unpredictable.
- ***Genetic engineering***, on the other hand, allows not only related species to be mixed, but extends the genetic pool to species not even related to that particular target organism. A preliminary scan of distinct properties tracks down certain genes that later on are isolated and extracted from the source-organism. By inserting the extracted gene into bacterial plasmids (vector), the genetic sequence is shipped into the target plant – a process that is more efficient and less time consuming.
  A widely used bacteria used for this purpose is *Agrobacterium tumefaciensis*; due to its large plasmid, it is capable of inducing tumors (uncontrolled growth) in plants. GM-engineers use a restriction enzyme to extract the plasmid and insert it into the plant seedling – at present only single genes or single groups of genes can be inserted this way. Herbicide and disease resistant plants can be generated this way.
  Another approach is to generate an entire variety of plants (new species), like the one termed "***SBGE-crop*** (sky-blue glow effect). In this case, the plant has been modified with a bio-luminescent gene of a marine jellyfish. In particular the *aequinon* encoding gene sequence is extracted and inserted into the plant. The result of this modification is an on-site indication when the plant is experiencing stress, in that the plant starts to emit a bluish light - the stronger the stress the more light is emitted – in this way it indicates drought, cold, nutrient insufficiency, etc. (reason: plants under stress contained higher contents of calcium and the *aequinon* is used to detect this higher calcium concentration by triggering the light emitting cascade).



Ecosyl, developed by ICI-UK, is yet another successfully applied modified product. The grass to feed the cows over the winter naturally contains wild strains of bacteria; the bacteria utilize the grass' sugar content and converted it into acid; this is a randomly controlled process, resulting in huge variations from one silage to the next. By injecting ecosyl into the silage, however, the modified *Lactobacillus plantarum* not only converts the sugar more efficiently, but also makes it more palatable for the cows. Ecosyl grows very fast and competes extremely well with the naturally occurring strains and keeps the grass' pH at a fairly constant level. ICI launched even an improved version of it (**ecobale**) that inhibits the formation of molds while grass is stored in bales on the field.

Breeding disease resistant plants: This is mainly achieved by a *gene transferring* method. Again *Agrobacterium* is used to make them resistant to virus, fungi, etc.; in tomatoes, tobacco plants (to make them resistance against the *mosaic* disease) and many other vegetables.

- Once the protective **gene sequence** is inserted into a plant, it is able to block the pathway of the pathogen's metabolic synthesis (comparable to a negative feedback loop described at p 3).
- **Protoblast fusion**: a technique in which the plant's cell walls are dissolved under aseptic conditions and fused with another cell to generate a heterokaryonic cell; upon triggering vegetative reproduction, the lump of cell mass is then placed onto a cultivation dish (usually agar) to obtain the new organism; the newly combined genome results in a plant with properties of both genotypes; besides disease resistance, the plant can be enriched with genes that encode for growth regulations, frost resistance, etc.

Growing insect resistant plants: Insects can destroy crops in a matter of hours (e.g. the African locus) ; therefore, many ways have been chosen to pose a barrier to such invaders:

1. **Broadband insecticides**: Insecticides like DDT, that do not readily decompose, affect not only insects, but accumulate in plants and ultimately in humans. DDT remains for decades in the substrate, even though it does not stick to plants permanently but is easily washed off into the drinking water - unfortunately, DDT is still widely used in less developed countries, even though it has been banned by the WHO.
2. **Narrow-band insecticides**: insecticides that only affects closely related insects or a particular species.
3. **Selectively working insecticides**: *Bacillus thuringiensis* is a very versatile bacterium; under certain conditions, it changes into the sporulation phase. If this spore is ingested by an insect, the GIT-enzymatic breakdown liberates a spore-containing a toxin that ultimately kills the insect – in this way both the larvae, the carterpillar, as well as the adult insect are affected. However, only the animals with the correct enzymes are susceptible to this spore. So far, this spore does not survive well in all environments – even though scientists are already working on that. It is usually applied as a powder where it can survive for several days on the plant. In tomatoes for example, the genes of the bacterium are even passed on to the next generation. Monsanto's tomatoes, besides having an extended shelf life, are resistant to insects.

Genetically engineered farm animals: The **tick resistant sheep** (as the thick sucks blood, the modified blood plasma containing chitinase is able to break down the thick's chitin shell) and the turbo cow **Hermann** are very prominent example.

The **Turbo** or **BST cow,** as it is also known, contains a gene that encodes for the Bovine somatropin hormone. *Somatropin* is a naturally occurring polypeptide which is secreted by the pituitary gland; it acts as a growth hormone . When BST is injected into cows, it makes them also to produce 25 % more milk in which all fats from the body are deviated for the milk production. To obtain this result, farmers have to inject BST on a regular basis.

The biotech industry isolated the gene-sequence coding for BST and inserted it into a plasmid, and vectored into *E.coli* for rapid multiplication. Artificial BST lacks extra amino-acids, if injected into cows, they will require extra food to cope with the increased milk production.

	Food	**Increase of milk production**	**Ratio of milk vs. food**
No BST	34	28	0.8
BST	38	37	≈ 1

There are some serious side-effects of injected BST in cows:

Prolonged use of BST lowers the cows immune system BST - they have a 78% higher chance of getting sick; they easily get more infections, so far there are no other observable side-effects like birth defects, or changes in behavior. Nevertheless, in 1993, after a 7 year study, the FDA approved it.

- Milk of untreated cows contains about 2 ppb BST
- BST treated cows have a content of 10 ppb BST

The EU on the other hand, has asked for more clinical studies, since they fear that BST may have effects on humans. It seems that the polypeptide tends to break down in the GIT of humans, but there are still traces of it found in lactating mothers. Furthermore, there are still concerns regarding the animals immune system.

Single Cell Protein (**SCP**): Proteins derived from microbial cells for use as food or food supplements; SCP's are biotech's real success story as these proteins are usually obtained from so called "*waste*" substrates, and are widely used in the UK and USA.

Applications of SCP:

- **Cheese** industry: the huge amounts of whey that otherwise are dumped as sewage (can contaminate drinking water), are further processed by GM-yeast that use up the remnant lactose, proteins, and vitamins. Nowadays, the resulting product is used as food supplements for cattle.
- **Sugar** industry: molasses the end product in sugar refinery used to be fermented for rum-making, nowadays it is further processed by GM-bacteria to obtain a cattle food supplement.
- **Sulfite liquor**: a waste product of paper mills contains low levels of sugar; feeding GM-fungi with this low-level nutrient, yields bio-degradable waste while generating substantial amount of heat that can be further used.
- **Natural gas** used to be flamed off in cracking towers; with the help of GM-methanous bacteria, this gas can be converted to long-chain hydrocarbons; i.e. liquid fuel;
- **Alkanes**: straight chained alkanes are a waxy oil; until the dawn of biotechnology there was very little use for them; the biotech industry came up with a GM-modified bacteria that converts alkanes into primary substrates for the synthetic protein production of another GM-branch.

Advantages of SCP: microorganisms are R-strategists (i.e. grow and multiply rapidly); some microbes are able to double their biomass up to three times an hour:

- microorganisms can exploit a great variety of (waste) substrates - represents a huge profit margin;
- the genotype of microorganisms can be manipulated very easily; screening of the manipulated organisms is facilitated by their rapid growth rates;
- microorganisms in relation to their size, are not only rich in proteins, but also in amino-acids;
- providing the warmth and ideal growth conditions, they can be easily accounted for in a fermentor;
- furthermore, fermentors do not require that much space as conventional techniques in agriculture; i.e. spatial requirements for farms and ranches.

Disadvantages of SCP: a very serious side-effect in the production of SCP, is the high percentage of contaminants that can be found in the extract (traces of sewage, excrements, waste, etc); purification at this stage is rather expensive and no one can guarantee a 100% pure product; therefore, SCP's are economically not yet profitable enough. An alternative to this handicap may be the implementation of a food-chain; i.e. the toxic raw substrate is used up by organisms A; then second organism B utilizes organism A as its substrate; ultimately only the end-product of organisms B is used in the harvest of SCP.
Even though the final product is free of contaminants, there are some setbacks in this approach:

- food-chains are usually less efficient (energy losses) - roughly 10-30% is obtained at the end;
- this is also reflected in the low nutrient level and higher financial costs;
- another important aspect has to be taken into account; as mentioned before, prokaryotes contain a large amount of ***nucleic acids*** in order to maintain exponential growth; compared to other foodstuffs, the SCP's nucleic and amino-acid content is around 15%, while those in conventional food is only around 4%; consequently, relying on SCP's only would cause *serious health problems*, like kidney stones, diarrhea, vomiting, etc. SCP's can therefore, only be utilized as food supplements for humans and animals. An option to this dilemma can be seen in the introduction of eukaryotes like fungi, that contain far less of it;
- contamination with pathogens can never be excluded; theoretically there shouldn't be any, practically there are always some - even in conventionally processed foodstuffs; heat-sterilization may kill these germs, but will also affect the generated proteins resulting in the type of food that is generally known as ***junk food*** - products that have very poor nutrient levels.

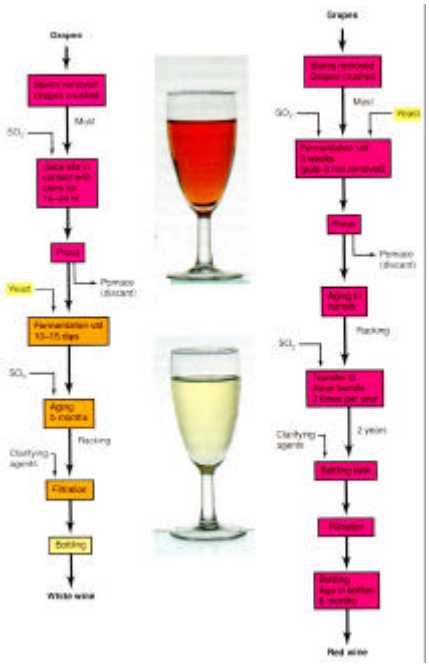
Selected items made of SCP's:

- **Marlow food** an item belonging to the instant food products made of GM-fungi, that is very easy, fast, and convenient to cook. The success story is that the fungi, *Fusarian gramineaum*, has lots of fibers that can be arranged to look like meat - a similar product is known under the brand name Qorn.
- **Health Care Products** - the GM-autotrophic blue-green algae *Spirulina sp*., is used as a food additive; it has a very high nutritional value and is most appreciated by health conscious individuals.
- **Prutein** another promising food supplement was developed by ICI; it was intended to be used as a high protein food for cattle - as it was launched, farmers seem to like it. In 1972 prutein was the largest investment of ICI, then fish meal came onto the market - as fishmeal outcompeted ICI's supplement, they were forced to completely shut down production of this GM-product all together - there are chances though, if fish stocks dwindle that they may relaunch production again.

- The **wine making** industry used to rely on traditional methods; such wine is made of grapes that contain different strains of wild yeast that can be found on the grape's skin. Different strains of yeast give the same wine a different flavor according to which strain of yeast dominates the season's harvest and which geographical area the grapes are cultivated in.

  Nowadays, vineyards tend to cultivate their yeast strains separately to guarantee that every harvest yields the same unique taste that consumers might look for.
  To understand how this is done, a quickstep recipe shall explain the basic steps (these reactions are carried out in huge stainless steel fermentation tanks):

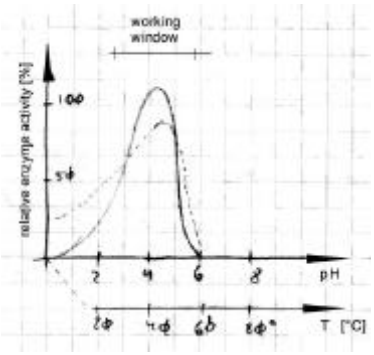
  1) To kill the naturally present strains of yeast (usually strains of *Saccharomyces cervisiae*), the grapes of the first mast are mixed with sulfur dioxide (SO_2); at the same time, this procedure also halts the beginning of fermentation process;
  2) upon neutralization of the acid, inoculation of the mast with the designed GM-yeast starts the actual fermentation, which ultimately, yields white wine;
  3) to obtain **red wine**, the prefermented mast is mixed with tiny amounts of dark-colored grape-skin; the already present levels of ethanol dissolve the reddish pigment and color the mast accordingly.
  4) within the 1st year a second fermentation with *malo-lactic acid?* reduces the acidity of wine;
  5) to **soften the taste** of the wine, a culture of *Pediococcus* bacteria is added to the mast;

  i) the production of **sparkling wine**, like champagne, requires further, alcohol fermentation; adding extra amounts of sugar into the tightly sealed fermentor, creates a fair amount of CO_2, which becomes dissolved in the champagne if pressurization is maintained; to obtain different brands (flavors) of Champaign, the mast is enriched with selected blends of red or white wine;
  i) **Sherries** are made by increasing the alcohol content to 15 % (adding extra ethanol); in addition, the fermentation process has to take place under atmospheric conditions - exposition to air creates a thick film of yeast that floats on top; the end product possesses the characteristic flavor of the yeast; again, to maintain a typical Sherry's taste, most often producers expose their mast to GM-modified yeast cultures;
  i) **Dessert wines** have to be extensively exposed to microbial treatment with a GM-fungi *Botrytis sinerae*; the fungus causes not only dehydration of the grapes (loss of water) but also destroys the malic acid, rendering the wine less acidic while boosting the wines sugar content. Adding GM-yeast to the sweet mast, yields an end product that has a high level of fructose.

- In conventionally made **fruit juices**, the fruits are harvested, pressed and bottled. A profitable large scale production required year round supply of fruits; fruits stored in a cool and CO_2-rich environment, tend to loose part of their sugar contents. To boost the levels of sugars upon making of the pulp (crushing phase), extra GM-enzymes (e.g. pectinase) are added; these enzyme cleaves the pectine of the fruit, and lowers the water-binding capacity of hemicellulose (weakening of the cell walls which extracts more liquid out of the fruit); enlarging the manifold types of juices; the industry is able to produce juices of fruits that have been unthinkable before.

  A cocktail of **tailored enzymes** are used specifically for citrus juices like oranges and grapefruits, while other cocktails are designed for berries, stone fruits, etc. The production of juices, even with those enzymes, require precisely adjusted technical equipment; an optimal working window (mostly in terms of temperature and pH) in order to maximize output, while not causing the denaturation of enzymes or destruction of vitamins.

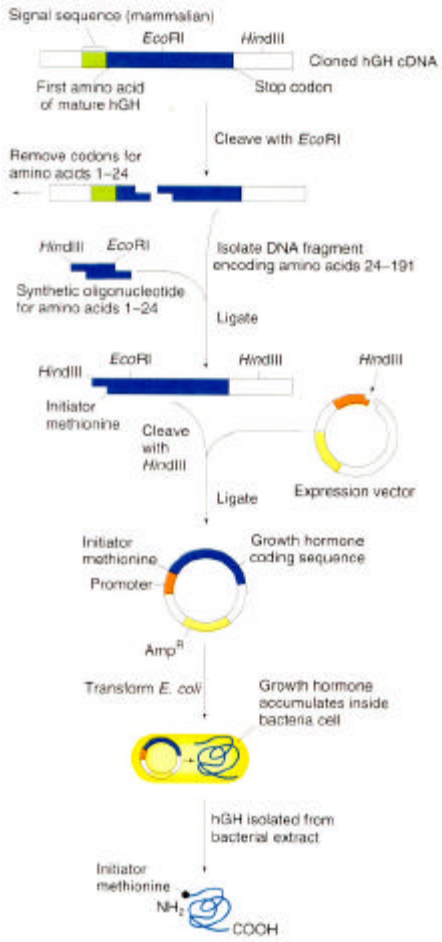
  

  The particular color of a juice originates from the berry; i.e. according to the geographical location; to maintain both color and taste of berries of various locations, the designed enzymes have been modified in a way that they provide high yields and the preservation of color, flavor and aroma of the product.
  Many juices are advertised as containing "*no added preservatives*" or "*no colors and flavor enhancers*", because these enzymes along with the proper temperature are designed to achieve that task - for the joy of the fruit juice consuming public.

Part IV - Biotechnologie and Health Care - in a broader sense caring about people not the medical aspect;

Human protein production: today many products are made artificially by introducing gene sequences into a cell (i.e. bacteria, yeast, or other microorganisms); ***interferon*** via bacteriophages, ***interleukin*** via bacteria, etc are one of those examples that are manufactured in this way.

- The **human growth hormones** by conventional means has been extracted from human plasma - it was a major pathway of CJD (Creutzfeld Jacob disease) transmission. If this hormone from the pituitary gland, is present in reduced quantities in children that may suffer from dwarfism (one mayor cause of its absence in growing adolescence has been detected in the lack of GH-releasing factor that originates in the hypothalamus). Today, recombinant gene technology uses bacteria in order to produce it on a large scale; and technology seem to work so well that dwarfism may be overcome in few years time.
- **Insuline** controls the blood-glucose levels; patients suffering with diabetics have an insufficient supply of this hormone. Insulin used to be extracted from the pancreas of cattle and pigs - often such insulin was the cause of allergic reactions in many insulin users. Biotechnology made it possible to produce insulin on a grand scale by simply inserting the corresponding gene sequence into a bacteria. Such insulin resembles very closely to that one secreted by the human pancreas - thus it has very few side effects.
- **Factor VIII** is very familiar to those people who suffer from hemophilia; again with the help of biotechnology, this factor is produced by bacteria; it has greatly reduced the likelihood of hemophiliacs to contract AIDS, as previously applied substances originating from blood-plasma donors.



- **Erythropoitin** is a hormone produced by the kidneys; it stimulates the production of red blood cells (erythrocytes). Patients with kidney failure do not produce this hormone anymore; therefore, they often suffer from anemia, are always tired, and apart from dialysis, the need a constant supply of fresh blood transfusions.
  Today, this hormone is made by a transgenic mammal, of the *Chinese hamster*. Extracting plasma from the animal, isolating the hormone, is a safer way to obtain this hormone, rather than relying again on human donors.

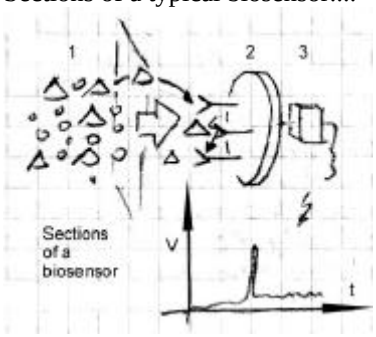
Biosensors are required to respond to changes in the environment; every living organism needs them for its survival, defense, to find food, to avoid danger, etc. Biosensors are usually very specific and sensitive (i.e. sense of smell in sniffing dogs, blood-tasting sharks, etc).

Characteristics of a biosensor:

1. Preselection: mixture of substances (antibody, enzymes) penetrates a selective permeable membrane (biorecognition layer), only certain molecules can pass it (pre-screening);
2. Layer of recognition: particular molecules bind to receptors (membrane component); upon contact, the receptor triggers a biochemical signal cascade; in biomolecules, this is usually an enzymes cascade that amplifies the signal.
3. Transducer: a chemo-electrical device (signal converter); the amplified signal results in a stimulus that is passed on to the processing center (i.e. brain); at this stage a response pattern is provoked (defense or attack reaction).

In electronics, a transducer can be either **potentiometric** (does not require an external power supply; e.g. pH-electrode) or **amperometric** (requires an external power supply; e.g. pO_2-electrode). Both transducer types generate an output voltage (ΔV).

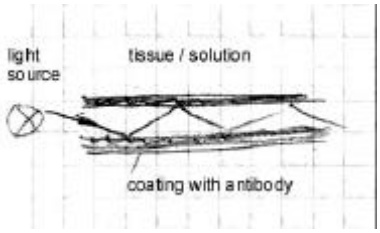
Sections of a typical biosensor....



....and its response pattern

Nowadays, **thermistors** are developed where the immobilized enzyme, releases tiny amounts of heat, that is detected and converted into an electrical signal; one of the first successfully applications (20 years ago) have been made with glucose detecting sensors; this was a major step forward, as surgeons were able to monitor the oxidation process of glucose upon exposition of blood to oxygen of the air.

Fiber-optical biosensors are a modern approach in that sector. Upon insertion of the tiny fiber into the human tissue and irradiation with visible light, the refracting power of the glass-tissue-blood interface changes drastically; as the outside of the fiber is coated with an antibody recognizing sheath, any bonds of an antigen with an antibody will not only further alter the refracting powers but also results in fluorescence, thus completely altering the reflected pattern of light; a transducer converts the changed pattern of this reflected beam into an electrical signal.



Antibody coated optical fiber

Microchip-biosensors: this approach requires a microchip with a built-in sensor and transducer (i.e. glucose detector); as glucose oxidizes upon exposure to air, the resulting gluconic acid can easily be detected by the sensor; the concentration of this acid is directly proportional to the resulting transducer current. Futuristic concepts even suggest that implanting such sensors into the human body might alert the person even before damages occur.

Similar **biochips** are already developed by IBM; (won't be larger than 2 mm in diameter); it will have the advantage of not only detecting the signal but also capable of processing that signal in order to suggest / launch counter measures. This chip is supposed to be self contained, small, robust, responsive to any signal; it is intended to be implanted into the skin, acting as an artificial control mechanism; i.e. in the case of an artificial pancreas: control, measure, and dispenses of the right doses of insulin into the blood stream.

The use of biosensors to monitor the **Quality of food** represents a powerful tool; the meat industry would have a reliable method to monitor the quality of the food product, its proper storage (interrupted cooling chain), in that the sensor provides immediate data for the contamination with toxic microorganisms (*Salmonella*, etc.) - a direct indicator of how fresh is fresh**!**

These biosensors suppose to provide on the spot and accurate results for:

a) Fermentation reactions: how much sugar, metabolic waste products are built up by microorganisms, pH, nutrients, etc.
b) Detection of bacteria in fast food.
c) Environmental monitoring, in which microorganisms are used to detect explosives, toxics, gasses, etc. and even in forensic science.

Potential risks of biochips:

- Checking on the health status of the employees; biosensors in toilets to monitor the urine of employees, (sugar levels, instant pregnancy tests, etc) for the projection of long term employment status.
- Monitoring sweat of people (against stealing) - everywhere in which employees might take advantage of the company's property for their private use.
- In the insurance industry, to exclude certain individuals from insurance packages, etc.

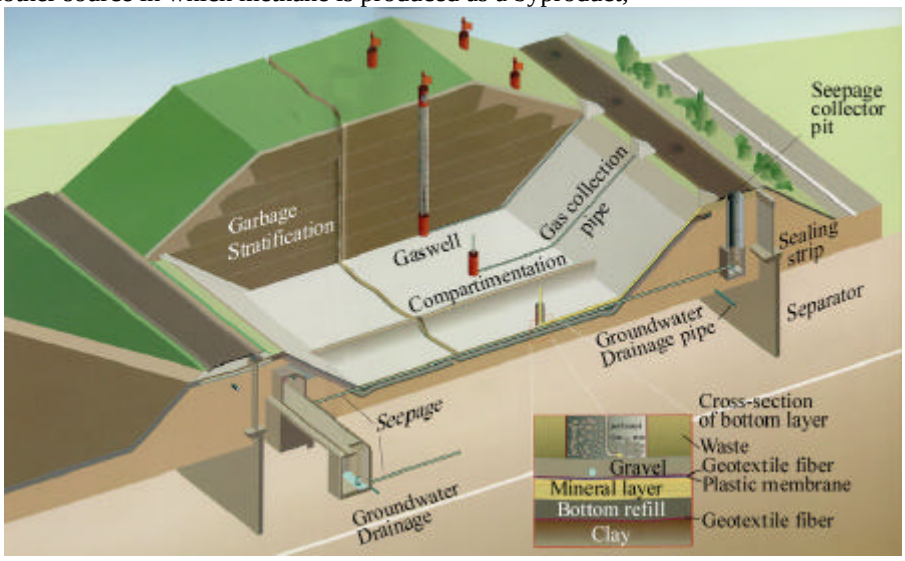
Part V - Biotech and the manufacturing industry

An **enzyme** is a catalysts that accelerates the rate of a specific chemical reaction (substrate and product specific - the substrate-protein complex is very specific).
Dr Otto Rohm was one of the first to realize this concept; in those days, at the turn of the last century, leather factories used feces and excrements of humans and animals to soften and de-hair furs; by carefully analyzing the procedure, he found out that enzymes of the pig's pancreas can be useful substitute.
Today, enzyme-mediated production methods not only lowers the pressure, temperature, and the amounts of toxic by products, but also lowers operation costs. The major disadvantage, though, is that enzymes are proteins, they tend to denature at high temperatures and extreme pH's. Therefore, what the industry is looking for, are GM-enzymes that are robust enough to withstand all industrial working conditions; such specialists are found among the thermophilic, acidophilic, and barophilic archaeal bacteria.
These microorganisms offer all the answers to all the problems that eubacterial enzymes do have; some successful implementations have already been made:

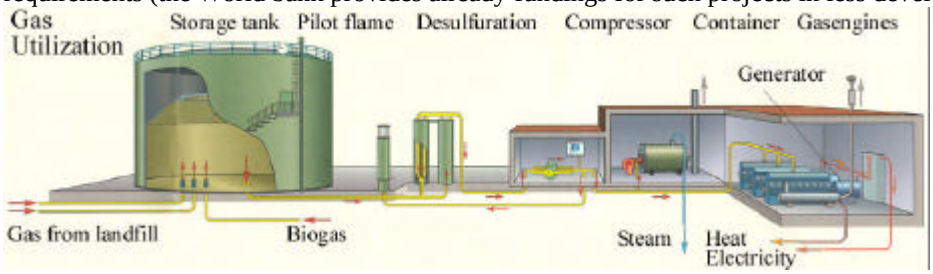
- To break down starch and food-marks on clothing, a modern **washing powder** requires basically two enzymes (amylases and proteases); these enzymes are capable of removing organic stains like egg, blood, meat, etc.; There are some complications to be aware of; the workers at the manufacturing plant were showing allergic reactions to those enzymes; as a consequence, these enzymes had to be encapsulated to protect the employees and the manufacturer from compensation payments.
- The **confectionery industry** utilizes the fermenting power of *Bacillus subtilis*, *Aspergillus niger* in order to save on expenses for sugar - they use these organisms to make glucose syrup out of potato starch.
- In **agricultural forestry** the use of *Sporotrichium* substantially reduces the previously useless piles of wood chips. This microorganisms cleaves the cellulose but not the protein. The remaining lignin can then be mixed with glucose and used as a food supplement in the cattle industry.
- The **textile industry** always tried to produce smooth and soft fibers that are pleasant to wear; but at the same time they should withstand elevated temperatures during washing cycles. The industry came up with a GM-bacterially made amylase; similar to the detergent's trick, they use amylase to remove the starch and at the same time render the fabric smooth and resistant to boiling temperatures.**Methane production with the help of biotechnology**: Conventional CH_4 production were achieved via the anaerobic decay of organic matter (animal and plants); this technique is well known and is becoming wide-spread throughout the world - it is of essential importance for the less developed countries to lower their dependence on fossil fuel energy sources.

The classical production of CH_4:

1. To reduce the water content of **sewage**, it is stored in an anaerobic digester (solid-liquid phase separator); subsequently attached aerobic treatment in which microorganisms are added, generates a sludge that is self-containing (doesn't require an external heat source) and produces the useful byproduct methane - part of this gas is used to power the sewage plant itself.
2. **Urban landfills** are another source in which methane is produced as a byproduct; CH_4 production in that way was even known in roman times; and it is becoming even important nowadays in which huge amounts of rubbish that occupy large areas of land worldwide. Collecting the methaneous gasses and storing them in tanks provides an other source in which this energy can be implemented in our daily lives.



3. **Agricultural fermentors** are another source of biogas production and can be of essential importance in rural areas where other forms of energy are rare - especially in developing countries; it requires animal and/or human dung, as well as any organic material like residue of crops. Adding water to it generates an almost anaerobic conditions; the produced biogas (mix of CO_2 and CH_4) can be used as fuels, while the fermented sludge are rich in nutrients (amino-acids, phosphates, nitrates, etc). Although building costs are quite high, such facilities in the long run are far cheaper than relying on conventional fossil fuels. Furthermore, biogas can also be used to power equipment and generate electricity for the owners requirements (the World bank provides already fundings for such projects in less developed countries).



4. **Gasohol**, or liquefied coal, is known already since the 1890, and widely used during WWII. Nowadays, gasohol is made of oils from crops. Brazil is one of the countries in which gasohol is a common fuel substitute. They use sugar cane, casaba-roots, cellulose wastes, tequila, palm wine and other cheap raw materials, for alcohol distillation. Brazil is becoming one of the first countries that in combination with genetic engineered microorganisms are capable to reduce their dependence on fossil fuels, their financial budget and also CO_2 emissions.

Mining and Biotechnologie: Some bacteria are very efficient in the extraction of metals from low grade ore (< 10 %); certain chemo-lithotrophic microorganisms are able to reduce metallic ore to their elemental form. Old waste piles of mines in combination with GM-bacteria are used to extract low levels of metal still enclosed in these ores. *Thiobacillus* derives its energy from thermo-autotrophic behavior; as these strains withstand low pH, extreme temperatures, etc, they are used to extract Co, Ni, Pb, and other metals from low grade ore. Further advances in genetic engineering can help facilitate mining by creating new ways of metal extraction that are less harmful to the environment and of course far cheaper than conventional techniques.

- **Copper mining**: since the 17th century it is known that water that drains through a pile of copper ore extracts copper in its elemental form. This moisturizing effect of water enables the *Theobacillus* sp to oxidize the sulfides to H_2SO_4, while reducing the metal (as observed in Spain with pyrite). Already 25% of all copper mines obtain their metal with the help of microbial activity.
- **Uranium Mining**: in the 1940's scientists discovered underground lakes that were enriched with radioactive uranium; again it was *Thiobacillus* sp. that has extracted the metal from the surrounding ore. Modern uranium mines have picked up this way of metal extraction - not only it protects humans from the dangers of radiation, but also trims down the risks to the environment. Even though the use of uranium remains controversial, mines in Brazil, Australia and in South Africa rely on this method.

Oil Industry: So far even the most efficient oil extracting technology is only capable of recovering roughly 50% of the oil from the wells; the rest lies dormant underground. Biotechnology seems to enhance this very low level of efficiency by using microbially mediated recovery technologies with the aim to extract the remaining amounts of oil still stored underground.

1. **Microbial enhanced recovery** (MER): In this approach, microorganisms are injected into the wells; as these organisms reduce the surface tension (viscosity), the trapped oils drip out as a liquid (fluid). A commonly applied product is **Emulsan**, a polysaccharide that specifically acts on certain hydro-carbons. **Xanthangum** a product obtained from *Xanthomonas sp*. is a viscosity-enhancer, that is mixed with water and thereby flushing out the residual oils.
2. Biotechnology also helped in limiting the damaging effects of oil-spills to the environment. Oils from accidental spills are exposed to certain GM-strains of *Xanthomonas* that are able to feed on it. Every strain is able to digest a distinct type of hydro-carbons (pentanes, hexanes, octanes, etc.). Scientists are already working on a ***superbug***, that is capable of eating up all different sources of oils. During the Gulf War, such trials seemed to have provided promising results; there is a drawback though, these strains work only on the water-oil interface, which means, the sticky and very thick oil slick requires water, for the bacteria to break down the oils into smaller fragments - but oil is hydrophobic..... Other concerns regard the potential dangers of such a super bug - could it become an environmental monster?, would it be a species-friendly bug?. Questions that still awaiting answers, uncertainties are still prevailing, and for now it is better to leave it as it is.... nevertheless, scientists intensively work on a form of that strain that can be pressurized and sprayed onto an oil slick.....